CONCLUSION

Claims 1-11, 14-27 and 34-36 were pending in the application. Claims 1, 11 and 35 are amended by the present submission.

Applicants respectfully request continued examination of the application in light of the amendments and remarks made in the submission. If the Examiner believes that a telephonic interview would expedite the allowance of the application, the Examiner is invited to contact the undersigned attorney at the number below.

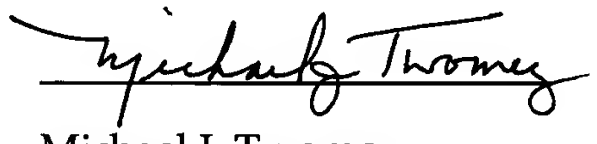
Applicants submit herewith a Petition for Three Month Extension of Time. The Commissioner is hereby authorized to charge the fees for the Three Month Extension of Time and for the Request for Continued Examination to Deposit Account No. 08-0219.

Respectfully submitted,

WILMER CUTLER PICKERING

HALE AND DORR, LLP

Date: June 18, 2004



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APPENDIX A

Copy of the Response to the Final Office Action dated December 19, 2003 that was filed on March 10, 2004.

COPY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Jakobsen *et al.*	Art Unit:	1644
Serial No.:	09/334,969	Examiner:	M. DiBrino
Filing Date:	June 17, 1999	Conf. No.	5926
Docket No.:	102286.410	Cust. No.:	23483
Title:	**Multivalent T-cell Receptor Complexes**		

CERTIFICATION UNDER 37 C.F.R. § 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450 on the date shown below.

3/10/04
Date of mail deposit

Sharon L. Matthews
Sharon R. Matthews

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT AND RESPONSE PURSUANT TO 37 C.F.R. § 1.116

Sir:

In response to the Final Office Action dated ***December 19, 2003*** (hereinafter, "Office Action"), please amend the above-identified application as follows:

Amendments to the Claims begin on ***page 2*** of this paper.

Remarks/Arguments begin on ***page 6*** of this paper.

Reconsideration of the application in light of the foregoing is respectfully requested.

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of claims:

Claim 1. (Currently Amended): A synthetic multivalent T cell receptor (TCR) complex for binding to a MHC-peptide complex, which TCR complex comprises a plurality of T cell receptors specific for the MHC-peptide complex, wherein each TCR in the complex is a refolded recombinant TCR which comprises:

i.) a recombinant TCR α or γ chain extracellular domain having a first C-terminal dimerization peptide which is heterologous to the α or γ chain; and

ii.) a recombinant TCR β or δ chain extracellular domain having a second C-terminal dimerization peptide which is heterologous to the β or δ chain and which is specifically heterodimerized with the first heterodimerization peptide to form a heterodimerization domain,

wherein a disulfide bond present in native TCRs between the α and β or γ and δ chains adjacent to the cytoplasmic domain is absent from the recombinant TCR.

Claim 2. (Original): The TCR complex according to claim 1, wherein the T cell receptors are αβ T cell receptors having an α chain and a β chain.

Claim 3. (Original): The TCR complex according to claim 2, wherein the α chain and β chain are soluble forms of T cell receptor α and β chains.

Claim 4. (Previously Presented): The TCR complex according to claim 1, wherein the T cell receptors are in the form of multimers of two or more T cell receptors.

Claim 5. (Original): The TCR complex according to claim 4, wherein the multimer is a trimer or a tetramer.

Claim 6. (Previously Presented): The TCR complex according to claim 1, wherein the T cell receptors are associated with one another via a linker molecule.

Claim 7. (Previously Presented): The TCR complex according to claim 6, wherein the linker molecule is a multivalent attachment molecule.

Claim 8. (Previously Presented): The TCR complex according to claim 7, wherein at least one of the T cell receptor α or β chains is derived from a fusion protein comprising an amino acid sequence encoding a protein tag.

Claim 9. (Original): The TCR complex according to claim 8, wherein the T cell receptors are biotinylated.

Claim 10. (Previously Presented): The TCR complex according to claim 1, comprising a multimerized recombinant T cell receptor heterodimer having enhanced binding capability compared to a non-multimeric T cell receptor heterodimer.

Claim 11. (Currently Amended): A multivalent TCR complex comprising a multimerized recombinant T cell receptor heterodimer having enhanced binding capability compared to a non-multimeric T cell receptor heterodimer, wherein each TCR in the complex is a refolded recombinant TCR which comprises:

i) a recombinant TCR α or γ chain extracellular domain having a first C-terminal dimerization peptide which is heterologous to the α or γ chain; and

ii) a recombinant TCR β or δ chain extracellular domain having a second C-terminal dimerization peptide <u>which is heterologous to the β or δ chain and</u> which is specifically heterodimerized with the first dimerization peptide to form a heterodimerization domain,

wherein a disulfide bond present in native TCRs between the α and β or γ and δ chains adjacent to the cytoplasmic domain, is absent from the recombinant TCR.

Claims 12-13. (Canceled)

Claim 14. (Previously Presented): The TCR complex according to claim 11, wherein the heterodimerization domain is a coiled coil domain.

Claim 15. (Previously Presented): The TCR complex according to claim 14, wherein the dimerization peptides are c-jun and c-fos dimerization peptides.

Claim 16. (Previously Presented): The TCR complex according to claim 11, comprising a flexible linker located between the T cell receptor chains and the heterodimerization peptides.

Claim 17. (Previously Presented): The TCR complex according to claim 1, wherein the T cell receptor is expressed in an *E. coli* expression system.

Claim 18. (Previously Presented): The TCR complex according to claim 1, wherein the T cell receptor is biotinylated at the C-terminus.

Claim 19. (Previously Presented): The TCR complex according to claim 1, wherein the T cell receptors are associated with a lipid bilayer.

Claim 20. (Original): The TCR complex according to claim 19, wherein the lipid bilayer forms a vesicle.

Claim 21. (Original): The TCR complex according to claim 20, wherein the T cell receptors are attached at the exterior of the vesicle.

Claim 22. (Previously Presented): The TCR complex according to claim 20 or claim 21, wherein the T cell receptors are attached to the vesicle via derivatized lipid components of the vesicle.

Claim 23. (Previously Presented): The TCR complex according to claim 19 or claim 20, wherein the T cell receptors are embedded in the lipid bilayer.

Claim 24. (Previously Presented): The TCR complex according to claim 1, wherein the T cell receptors are attached to a solid structure.

Claim 25. (Previously Presented): The TCR complex according to claim 1, further comprising a detectable label.

Claim 26. (Previously Presented): The TCR complex according to claim 1, further comprising a therapeutic agent such as a cytotoxic agent or an immunostimulating agent.

Claim 27. (Previously Presented): The TCR complex according to claim 1, in a pharmaceutically acceptable formulation for use *in vivo*.

Claims 28-33. (Canceled)

Claim 34. (Previously Presented): The TCR complex according to claim 1, wherein the heterodimerization domain is a coiled coil domain.

Claim 35. (Currently Amended): The TCR complex according to claim ~~33~~ 34, wherein the dimerization peptides are c-jun and c-fos dimerization peptides.

Claim 36. (Previously Presented): The TCR complex according to claim 1, comprising a flexible linker located between the T cell receptor chains and the heterodimerization peptides.

REMARKS

The Office Action dated December 19, 2003 states that the instant application was found to be eligible for continued examination under 37 C.F.R. § 1.114, and that Applicants' amendment filed July 25, 2003 was entered.

Claims 1 and 11 are amended to insert an omitted phrase and not in response to any rejection relating to patentability.

The Office Action states that because claims 33-35 were misnumbered, these claims were renumbered by the Examiner as claims 34-36 respectively. Applicants thank the Examiner for pointing out and correcting this inadvertent clerical error. In this context, claim 35 has been amended to correct the dependency of this claim on claim 34.

The Office Action further states that non-elected claim 33 is pending in the instant application. Applicants respectfully disagree with this statement, because claim 33 was cancelled in a Preliminary Amendment filed June 17, 1999 (Express Mail Label No. EL 110243543US).

Accordingly, claims 1-11, 14-27 and 34-36 are pending in the instant application.

Objection

The Office Action states that there is a spelling error on page 93, *i.e.*, "R fren s". Applicants have not been able to locate this spelling error. In particular, the substitute specification filed July 25, 2003, has only 92 pages. The marked-up copy of the specification has the correctly spelled word "References" on page 93. "References" also appears at page 80 of the clean copy of the substitute specification. Applicants therefore request clarification as to the specific location of the spelling error or a copy of the page bearing the error.

Rejections under 35 U.S.C. § 103(a)

(i) The Office Action maintained the rejection of claims 1-11, 14-18, and 34-36, under 35 U.S.C. § 103(a), as being unpatentable over WO 97/35991 in view of Golden *et al.* (*J. Immunol. Meth.*, **206**:163-9, 1997), O'Shea *et al.* (*Science,* **245**:646-8, 1989), Garboczi *et al.* (*J. Immunol.*, **157**:5403-10, 1996), and Schatz (*Biotech.* **11**:1138-43, 1993).

Specifically, the Office Action suggested that WO 97/35991 teaches soluble, recombinant divalent and multivalent (including tetravalent) analogs of heterodimeric proteins such as αβ TCR that possess enhanced affinity for their target molecules, wherein the αβ TCRs are associated via Ig linker molecules which may further comprise a toxin and/or may be further linked by association via avidin. Golden *et al.* is relied upon as teaching soluble heterodimeric TCR, comprising α and β chains, each chain comprising a leucine zipper which dimerizes, one with the other. O'Shea *et al.* is relied upon as teaching heterodimer formation through leucine zippers from c-fos and c-jun. Garboczi *et al.* is relied upon as teaching a soluble TCR without the interchain disulfide bond present in native TCRs, and that heterodimerization, refolding and antigenic specificity of the TCR do not require such an interchain disulfide bond, transmembrane segments or glycosylation. Finally, Schatz is relied upon as teaching biotinylation of proteins.

The Office Action states that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have made a recombinant TCR as taught by the combination of WO 97/35991 and Golden *et al.* without the disulfide bond, as taught by Garboczi *et al.* and further modified as taught by Schatz. According to the Office Action one of ordinary skill would have been motivated to combine these references because Garboczi *et al.* teach that the presence of a disulfide bond is not important for heterodimerization and refolding, and further in order to increase the yield of correctly folded soluble TCR molecules.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. A prior art reference must be considered in its entirety, *i.e., as a*

whole, including portions that would lead away from the claimed invention. Second, there must be a reasonable expectation of success. Finally, the prior art references must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicants' disclosure.

Applicants note that Golden *et al.* and Garboczi *et al.* are critical to the reasoning provided in the Office Action. If either, or both, of these references do not adequately support the interpretation stated in the Office Action, the entire objection is unsustainable. Applicants submit that upon a proper reading of Golden *et al.* and Garboczi *et al.* one of ordinary skill in the art would not find any teaching, motivation, or expectation of success to arrive at Applicants' claimed invention.

The Office Action states that Garboczi *et al.* teaches that the disulfide bond present in native TCRs is not required for heterodimer formation. Respectfully, Applicants firmly disagree with this characterization of the Garboczi *et al.* reference. Specifically, with respect to Figure 4, a SDS-PAGE gel, the Examiner states that:

> Garboczi *et al.* teach "As expected, under nonreducing conditions no disulfide-bonded heterodimer was seen" (especially page 5407, column 1 at lines 8-10), indicating that ... with regard to the native gel shift electrophoresis, the noncovalently associated heterodimer was not expected to be observed as a complex, but as separate chains. (Office Action, page 6, first full paragraph).

and that:

> It is the Examiner's position that the native gel shift electrophoresis assay under nonreducing conditions was used to assess the composition of the heterodimer, i.e., to confirm that the heterodimer was consisted of both α and β chains and not homodimers of one chain or the other, and to confirm that the TCR was correctly refolded to bind MHC/peptide. The absence of noncovalently associated heterodimer on native gel shift electrophoresis assay under nonreducing conditions is indicative that when the heterodimer is subjected to the nonphysiologic condition of having an electric field applied, the non-covalently associated heterodimer does not migrate as a unit. (Office Action, page 6, first full paragraph).

Applicants respectfully contend that the statement in Garboczi *et al.* that "As expected, under nonreducing conditions ***no disulfide-bonded heterodimer*** was seen" [emphasis added] addresses the fact that the "short-form" constructs run on the SDS-PAGE in Figure 4 ***did not***

form disulfide-bonded heterodimers "as expected" because these constructs do not contain the cysteine residues required to form the disulfide interchain bonds. Therefore, Applicants disagree with the Office Action's conclusion as to what the reference teaches because:

(i) the quote clearly refers to the ***lack*** of non-disulfide-bonded heterodimers; and

(ii) the data presented in Figure 4 relates to an SDS-PAGE gel and ***not*** a native gel as suggested in the Office Action. The SDS in this gel is, of course, expected to denature the TCR α and β chains. Therefore, Figure 4 provides no assistance in answering the question of the non-covalent association of the TCR chains under native conditions, one way or the other.

The Office Action also suggests that the gel filtration chromatography in Garboczi *et al.* would yield a non-covalently-linked heterodimer. Applicants respectfully disagree. Garboczi *et al.* teach:

> The refolded noncovalently linked αβ heterodimer elutes during gel filtration chromatography at a volume expected for a 40-kDa protein when compared with the elution of standard proteins (data not shown). The calculated molecular mass of the heterodimer is 50.2kDa. (top of left column of page 5407).

Applicants would like to point out that Garboczi *et al.* does not give the calibration data for the gel-filtration versus standard proteins. Furthermore, this reference provides no data regarding the elution of an inter-chain cross-linked heterodimer during gel-filtration. Data concerning the latter would have provided the ordinary skilled artisan with a benchmark for the elution of a ***true*** heterodimer. Without the calibration data, and without a true heterodimer benchmark, it is not possible for one of ordinary skill in the art to interpret the results quoted in Garboczi *et al.* However, it seems that the non-associated chains have molecular weights of up to 27.5kDa (*see*, Figure 4 of Garboczi *et al.*). 40kDa lies midway between 50.2kDa (the calculated molecular mass of the heterodimer) and 27.5kDa (molecular mass of the non-associated chains), so *prima facie* it was not a heterodimer that was being eluted. Actually, it is impossible to conclude from the gel filtration data just what was eluted at 40kDa, but the fact that the molecular mass of the eluted product is intermediate between the weights of the heterodimer and the monomers reasonably supports the view that there was some form of dynamic association and dissociation going on. This view is entirely consistent with the native

PAGE results depicted in Figure 5, which show that no non-covalently-linked TCR heterodimer is formed absent the MHC. The fact that Garboczi *et al.* asserts that the 40kDa fraction is the "refolded noncovalently linked αβ heterodimer" which elutes at 40kDa ***does not make it so***.

Taking the teaching of Garboczi *et al.* ***as a whole***, it would be readily apparent to one of ordinary skill in the art that the ***only*** evidence in this reference of the formation of a noncovalently linked αβ heterodimer derives from the observation from Figure 5 that the two TCR chains came together to bind the peptide-MHC complex. Figure 5 evidences that noncovalently associated TCR/MHC/peptide complex can form, but does not provide evidence for the stable association of the chains in the absence of the peptide-MHC complex.

Turning next to Golden *et al.*, Applicants respectfully submit that this reference ***teaches away*** from the present invention. Golden *et al.* describes the production of murine D10 T-cell receptor in *E.coli* as a secreted leucine zipper fusion protein. The heterodimeric TCR of Golden *et al.* (in which the TCR chains are disulfide-linked and have C-terminal leucine zippers) was detected by conformationally sensitive TCR-specific antibodies only under non-reducing conditions. Golden *et al.* teaches:

> Under reducing conditions, all three antibodies failed to detect sD10TCR-LZ protein (Fig. 5, lanes 4, 5, 6), implying that recognition by these conformationally sensitive antibodies **is dependent on disulfide bond formation**. (page 169, left column). [emphasis added].

Therefore, since this reference teaches that conformationally sensitive antibodies only recognize the TCR heterodimer when the disulfide bonds are intact, Golden *et al.* distinctly ***teaches away*** from modifying the TCR components as taught by Applicants.

Because there is no motivation to combine the teachings of Golden *et al.* with those of Garboczi *et al.*, and because there is no expectation of success to arrive at Applicants' claimed invention based on the cited references, Applicants respectfully submit that this rejection has been applied improperly, and request that this rejection be reconsidered and withdrawn.

(ii) Claims 1, 24 and 25 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 97/35991 in view of Golden *et al.*, O'Shea *et al.*, Garboczi *et al.*, Schatz and U.S. Patent No. 5,635,363.

WO 97/35991, Golden *et al.*, O'Shea *et al.*, Garboczi *et al.*, Schatz have been relied upon for the reasons discussed above. U.S. Patent No. 5,635,363 is relied upon to disclose soluble MHC/peptide tetramers which are biotinylated and multimerized with streptavidin or with avidin and which further comprise a light detectable label or an enzyme and which may further be bound to an insoluble support such as a bead, *i.e.*, a "solid structure", for the purpose of assay.

As explained above, at least one element of the two independent claims of the present invention, namely that the "***disulfide bond present in native TCRs between the α and β or γ and δ chains adjacent to the cytoplasmic domain, is absent from the recombinant TCR***," is simply not taught or suggested by any of the claimed references, either alone or in combination. In particular, careful review of the experiments of Golden *et al.* and Garboczi *et al.* demonstrate that these references ***teach away*** from removal of the disulfide bond. Thus, there is no teaching, suggestion or motivation to arrive at Applicants' claimed invention. Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

(iii) Claims 1, and 19-24 were rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 97/35991 in view of Golden *et al.*, O'Shea *et al.*, Garboczi *et al.*, and further in view of Ahmad *et al.*

The Office Action relied on WO 97/35991, Golden *et al.*, O'Shea *et al.*, and Garboczi *et al.*, as discussed earlier. Ahmad *et al.* is invoked to teach attachment of a biotinylated targeting antibody to the surface of a liposome containing biotinylated phosphatidylethanolamine by means of an avidin linker and to teach that liposomes containing lipid derivatives of polyethylene glycol have circulation times sufficiently long to allow for effective *in vivo* drug delivery.

As explained above, at least one element of the two independent claims of the present invention, namely that the "***disulfide bond present in native TCRs between the α and β or γ and***

δchains adjacent to the cytoplasmic domain, is absent from the recombinant TCR," is simply not taught or suggested by any of the claimed references, either alone or in combination. In particular, careful review of the experiments of Golden *et al.* and Garboczi *et al.* demonstrate that these references ***teach away*** from removal of the disulfide bond. Thus, there is no teaching, suggestion or motivation to arrive at Applicants' claimed invention. Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

CONCLUSION

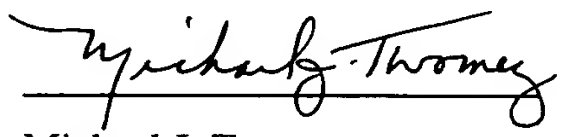
Claims 1-11, 14-27 and 34-36 were pending in the application. Claims 1, 11 and 35 have been amended by the present submission.

Applicants respectfully request reconsideration of the application in light of the amendments and remarks made herein. If the Examiner believes that a telephonic interview would expedite the allowance of the application, the Examiner is invited to contact the undersigned attorney at the number below.

No fees are believed to be due in connection with this matter. However, if any fees are due, the Commissioner is hereby authorized to charge the requisite fees to Deposit Account No. 08-0219.

Respectfully submitted,
HALE AND DORR, LLP

Date: March 10, 2004



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